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Treatment of osteoarthritis with mesenchymal stem cells

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Osteoarthritis (OA) is one of the most prevalent joint diseases with prominent symptoms affecting the daily life of millions of middle aged and elderly people. Despite this, there are no successful medical interventions that can prevent the progressive destruction of OA joints. The onset of pathological changes in OA is associated with deviant activity of mesenchymal stem cells (MSCs), the multipotent precursors of connective tissue cells that reside in joints. Current therapies for OA have resulted in poor clinical outcomes without repairing the damaged cartilage. Intra-articular delivery of culture-expanded MSCs has opened new avenues of OA treatment. Pre-clinical and clinical trials demonstrated the feasibility, safety, and efficacy of MSC therapy. The Wnt/β-catenin, bone morphogenetic protein 2, Indian hedgehog, and Mitogen-activated protein kinase signaling pathways have been demonstrated to be involved in OA and the mechanism of action of MSC therapies.

osteoarthritis, mesenchymal stem cells, intra-articular delivery

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Osteoarthritis (OA) is the most common form of arthritis, a disease that can affect all the structures of the joints [1]. It was estimated in 2007 that more than 100 million people in China suffered from OA [2]. In adults, OA is the second leading cause of work disability, and the costs of OA have grown exponentially to astronomical figures over recent decades, accounting for up to 1%–2.5% of the gross national product of countries with aging populations, including the USA, Canada, the UK, France, and Australia [3]. OA is not only a process of erosion, but also describes an anomalous remodeling of joint tissues resulting from obesity, joint instability, or trauma [4]. The extensive pathologic changes in OA are identified as "joint failure", which includes degradation of the articular cartilage, osteosclerosis of the subchondral bone, retrogression of cruciate ligaments, hyperplasia of the synovium, degeneration of menisci, and hypertrophy of the joint capsule [5]. Many treatments have been advocated, but most resulted in dissatisfactory clinical results without cartilage repair [6]. The only pharmacologic therapy for OA recommended by the American Academy of Orthopedic Surgeons (AAOS) is non-steroidal antiinflammatory drugs (NSAIDs) or tramadol for patients with symptomatic osteoarthritis. Intra-articular injection of hyaluronic acid is strongly not recommended [7]. In recent years, *The New England Journal of Medicine* published a series of results of controlled clinical trials showing little effect of arthroscopic surgery for the treatment of OA [8–11]. Furthermore, common treatments including physical therapy, viscosupplementation [12], glucosamine and/or chondroitin sulfate [13], and acupuncture [14] have demonstrated modest to no clinical benefit when compared with placebo. Moreover, all these treatments are generally intended to decrease pain, maintain or improve joint function, and minimize disability, not to regenerate joint tissue.

Cell therapy by surgical autologous chondrocyte implantation (ACI) has been used to regenerate chondral lesions for more than 20 years [15,16], and clinical trials have confirmed the efficacy of ACI. However, clinical trials with

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contradictory outcomes have been published by different institutions when comparing ACI with other classical treatments, particularly microfracture. Knutsen et al. assessed 80 patients following either ACI or microfracture. At 24 months, both groups showed significant clinical improvement, and no significant differences were found between the methods in either histological quality of the regenerated tissue or clinical. However, microfracture was associated with better SF-36 physical component scores [17]. More recently, Saris et al. [18] evaluated the efficacy of microfracture and characterized chondrocyte implantation (CCI) in 118 patients. At 12 and 18 months, CCI demonstrated better structural repair as measured by histological evaluation. At the 36 month follow-up, CCI showed a significant improvement not only in the overall Knee injury and Osteoarthritis Outcome Score (KOOS), but also in the subchondral bone reaction [19]. Nonetheless, at the 5 year follow-up, although the clinical benefit was retained, the overall KOOS was not different between the CCI and microfracture groups [20]. Furthermore, ACI has several inherent disadvantages, such as chondrocyte dedifferentiation during culture that might result in fibrocartilage rather than hyaline cartilage formation [21], the two-stage surgical procedure may cause further cartilage damage and degeneration [22], and chondrocyte yields and their *in vitro* proliferative capacity decrease with age, especially in older patients [23]. Most importantly, ACI has been limited to focal cartilage defects caused by injury, while generalized cartilage loss in OA has been considered an exclusion criterion [15,16]. Recently, intra-articular injection of mesenchymal stem cells (MSCs) for the repair of joint surface lesions suggested the suitability of MSCs to replace autologous chondrocytes as the cell source for OA cartilage repair [24–26]. The advantages of intra-articular injection of MSCs for the treatment of OA include the simplicity and ease of MSC delivery, minimum invasiveness, and avoiding the potential disease transmission caused by the xenograft coverage used in ACI. In this review, we summarize the characterization of MSCs, differences between adipose-derived mesenchymal stem cells (A-MSCs) and bone marrow-derived mesenchymal stem cells (BM-MSCs), aberrant activity of MSCs in OA, preclinical and clinical data of MSC therapies for OA, and potential mechanisms of action, including the signaling pathways and tissue regeneration involved in the treatment of OA with MSCs.

1 Mesenchymal stem cells and their phenotypes

MSCs are precursors of connective tissue cells and were discovered by Alexander Friedenstein, who was the first to identify a group of fibroblast-like cells in the bone marrow of mice and guinea pigs with the capacity not only to differentiate into osteocytes, but also to form colonies from a single cell, referring to them as colony-forming units fibroblastic (CFU-F) [27,28]. In 1991, Arnold Caplan suggested naming these cells 'mesenchymal stem cells' because they were capable of differentiation into all the cells of mesodermal lineage [29]. The minimum criteria for MSCs include plastic adherence and *in vitro* tri-lineage differentiation to adipogenic, chondrogenic, and osteogenic cells [30]. Additional requirements include cell surface expression of CD105, CD73, and CD90 and the absence of the hematopoietic markers CD45, CD19, CD79, CD11b, and HLA-DR. Nevertheless, the absence of a single specific marker that defines MSCs remains a particular challenge. Recently, a key potential marker for MSC enrichment, CD271 or low-affinity nerve growth factor receptor (LNGFR), was discovered [31]. After purification, $CD271^+$ non-hematopoietic bone marrow cells appear to contain all the colony forming cells [32], and $CD271^+$ cells demonstrate tri-lineage differentiation potential [31]. An additional marker, CD146 or melanoma cell adhesion molecule (MCAM), was also reported [32]. In human bone marrow, CD146 is expressed by a subpopulation of $CD271^{bright}$ cells, and the main subgroup in adults is $CD271^{bright}/CD146^-$ cells, while the $CD271^{bright}/CD146^+$ subgroup is dominant in pediatric and fetal bone marrow [33]. Both $CD271^+/CD146^+$ and $CD271^+/CD146^-$ cell fractions express classical MSC markers, including CD73, CD90, and CD105, and have tri-lineage differentiation potential. Furthermore, CD146 expression *in vivo* is localized to the perivascular regions within CD271-expression cells, while endosteal CD271 expressing cells lacked CD146 expression [32].

In addition to bone marrow, MSC populations can be isolated from dental pulp [34], umbilical cord blood [35], synovial membrane [36], adipose tissue [37], placenta [38], skin [39], umbilical cord perivascular cells [40], skeletal muscle [41], Wharton's jelly [42], meniscus [43], breast milk [44], cartilage [45], ligament [46], and fat pad [47]. The different sources of MSCs and their different phenotypic properties are shown in Table 1.

2 Differences between adipose- and bone marrow-derived MSCs

Adipose tissue is both an energy reservoir and a complex endocrine organ containing adipose-derived mesenchymal stem cells (A-MSCs). A-MSCs were first described as adipocyte precursors by Frohlich in 1972 [48]. Further investigations demonstrated the stem cell-like plasticity of A-MSCs and their capacity to differentiate into cells of mesodermal origin, such as adipocyte, osteocyte, chondrocyte, and myocyte lineages [49]. A-MSCs are now considered an attractive source of MSCs because of the large numbers of cells that can be harvested with relatively little donor morbidity. Compared with BM-MSCs, A-MSCs are more easily cultured and grow more rapidly [50]. The main benefits of A-MSCs are that their proliferation and differen-

Table 1 Characterization and phenotypic properties of MSCs from different sources

Cells source	Character identification	Phenotype	Reference
Umbilical cord blood	Positive: CD13, CD29, CD49e, CD54, CD90 a-smooth muscle actin Negative: CD14, CD31, CD34, CD45, CD49d CD106	Rich in mesenchymal progenitors, similar to haematopoietic progenitors.	Erices et al. (2000) [35]
Dental pulp	Positive: CD44, CD29, CD106, a-smooth muscle actin Negative: CD14, CD34, CD45	Produces only sporadic, but densely calcified, nodules, and does not form adipocytes.	Gronthos et al. (2000) [34]
Synovial membrane	Positive: CD44, CD73, CD90, CD105, CD166, CD271	Stable, proliferative population with higher chondrogenic potential.	De Bari et al. (2001) [36]
Adipose tissue	Positive: CD73, CD90, CD105 Negative: CD31, CD34, CD45	Higher potential yields of MSCs with chondrogenic, adipogenic, ostseogenic, and myogenic potential.	Zuk et al. (2001) [37]
Placenta	Positive: CD90, CD105, CD166, CD49e, SH3, SH4, HLA-ABC Negative: CD31, CD34, CD45, CD49d, CD123, HLA-DR	Higher expansion potency compared to bone marrow-derived MSCs.	In't Anker et al. (2004) [38]
Umbilical cord perivascular cells	Positive: CD105, CD73, CD90, CD44 Negative: CD45, CD34, CD235a, CD106, CD123, SSEA-4, HLA-DR, DP, DQ (MHC II), HLA-G, Oct4	Normal, rapidly expandable, $MHC^{-/-}$ cells containing a subpopulation that exhibits a functional osteogenic phenotype and elaborated bone nodules.	Sarugaser et al. (2005) [40]
Skin	Positive: CD90, SH2, SH4, HLA I, CD44, CD49d, CD49e, CD49f, CD166, CD105, EGFR, PDGFR Negative: CD45, CD38, CD34, CD31	Effectively differentiate into neuronal precursors better than bone marrow-derived MSCs.	Shih et al. (2005) [39]
Skeletal muscle	Positive: NG2, CD146 Negative: CD144, CD34, CD31	Strong myogenicity potential with typical MSC surface marker patterns.	Crisan et al. (2008) [41]
Wharton's jelly	Positive: CD10, CD13, CD29, CD44, CD90, CD105 Negative: CD34, CD45, CD14, CD33, CD56, CD31, HLA-DR	Greater expansion capability, faster growth *in vitro*, and different cytokine secretome compared to bone marrow MSCs.	Troyer et al. (2008) [42]
Meniscus	Positive: CD90, CD105, CD166, CD44 Negative: CD34, CD45	Less activity compared to synovium or bone marrow MSCs.	Segawa et al. (2009) [43]
Cartilage	Positive: CD49e, Notch1, CD90, STRO‑1	Higher fibronectin affinity and stronger colony- forming efficiency.	Williams et al. (2010) [45]
Breast milk	Positive: CD44, CD29, SCA-1, SMA, vimentin, nestin Negative: r CD33, CD34, CD45, CD73	Normally proliferative, with chondrogenic, osteogenic and adipogenic activity and normal MSCs phenotypes.	Patki et al. (2010) [44]
Ligament	Positive: CD29, CD44, CD49c, CD73, CD90, CD97, CD105, CD146, and CD166 Weekly positive: CD106, CD14 Negative: CD11c, CD31, CD34, CD40, CD45, CD53, CD74, CD133, CD144, CD163	Lower ability in chondrogenesis, osteogenesis and adipogenesis compared with bone marrow MSCs. Higher activity in ligamentogenesis.	Steinert et al. (2011) [46]
Fat pad	Positive: CD13, CD44, CD90, CD105, CD29 Negative: CD271, STRO1, CD34, CD56	Highly proliferative, strong chondrogenic, osteogenic and adipogenic activities.	Khan et al. (2012) [47]

tiation potentials and telomerase are less affected by age than those of BM-MSCs [51].

A-MSCs and BM-MSCs have slightly different cell surface marker profiles. Most recently, characterization of canine A-MSCs and BM-MSCs identified that canine A-MSCs and BM-MSCs both demonstrated strong expression of CD29 and CD44, moderate expression of CD90, and were negative for CD34 and CD45. Oct3/4 and Sox2 were equally expressed in canine A-MSCs and BM-MSCs, while Nanog expression was 2.5-fold higher in A-MSCs than in BM-MSCs [52]. Markers of human A-MSCs include STRO-1, CD146, and 3G5, in which the 3G5-positive fraction demonstrated the greatest enrichment for CFU-F compared with the other sorted cell populations [53]. However, there are slight differences in the cell surface phenotype profiles between A-MSCs and BM-MSCs despite sharing expression of key markers CD9, CD10, CD29, CD44, CD90, CD105, CD117, CD146, and STRO-1 [54]. Human A-MSCs express CD49d (VLA-4), which is not expressed by BM-MSCs, while A-MSCs lacked the expression of CD106 (VCAM-1), which was expressed by BM-MSCs [54]. This reciprocal expression is quite interesting because CD106 is the cognate receptor of CD49d. Moreover, only a small fraction of *ex vivo* expanded human BM-MSCs express STRO-1, while most human A-MSCs express it [55].

The differentiation potentials of A-MSCs and BM-MSCs are also slightly different. Compared with BM-MSCs, A-MSCs have lower innate chondrogenesis, though high-dose combinations of growth factors (TGFβ2 and IGF-1) were capable of inducing comparable chondrogenesis in both cell types [56]. Recently, the impact of culture medium on the differentiation capacity of A-MSCs and BM-MSCs has been investigated. Human serum derivatives (thrombin-activated platelet-rich plasma or human platelet lysates) can lead to spontaneous osteogenesis in BM-MSCs and strong adipogenesis in A-MSCs expanded *in vitro* compared with fetal bovine serum-supplemented media [57,58].

The immunomodulative ability of MSCs is considered to be one of their most important properties. In response to inflammatory molecules such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (INF-γ), MSCs secrete a variety of growth factors and anti-inflammatory cytokines that feed back to many types of immune cell [59,60]. A-MSCs, like BM-MSCs, are negative for MHC class II molecules, CD80-B7, and CD40 [61–63]. By contrast, A-MSCs prohibit B cell proliferation, decrease

immunoglobulin production, and restrict B cell functions more significantly than BM-MSCs [64].

3 Atypical activity of MSCs in OA

Although various investigations have demonstrated an atypical or defective activity of MSCs during OA progression, the relationship between this atypical activity of MSCs and OA progression itself remains unknown. Murphy et al. [65] revealed that bone marrow MSCs isolated from end-stage OA patients exhibit deficient proliferation and differentiation potentials compared with BM-MSCs from healthy, age-matched controls. In addition to a diminished yield and proliferative activity in OA MSCs, they also show a modified differentiation profile with decreased chondrogenesis and adipogenesis and increased osteogenesis. These aberrant activities in OA MSCs can be rescued by supplementation of the culture medium with growth factors such as epidermal growth factor or fibroblast growth factor 2 [66,67]. Human periosteal MSCs from donors younger than 30 years show spontaneous chondrogenesis in culture, while older donors do not exhibit spontaneous chondrogenesis [68]. Trabecular bone MSCs sorted on the basis of CD271 expression revealed that OA MSCs exhibit an age-related proliferation deficiency *in vitro* [69]. In OA patients, the number of MSCs in the synovial fluid compartment is much greater than in samples from healthy joints, and the number of cells increased with the severity of OA progression [70]. Synovial fluid MSCs show greater chondrogenic capability than MSCs separated from bone marrow [71].

4 MSC therapy for OA

Murphy et al. [24] were the first to propose the direct, intra-articular delivery of MSCs for the treatment of OA in a goat model. OA was induced by medial meniscectomy and anterior cruciate ligament transection (ACLT) for six weeks, and 10 million autologous BM-MSCs with sodium hyaluronan were injected. Twenty weeks after injection, the degeneration of articular cartilage, osteophyte formation, and subchondral bone thickening were reduced in the cell-treated joints.

Since then, a great deal of attention has been focused on the intra-articular injection of expanded BM-MSCs on animal models of OA (Table 2). In a porcine cartilage defect model, seven million autologous BM-MSCs were injected into 8 mm long and 1 mm deep defects, and 6–12 weeks later, the cell-treated groups exhibited improved cartilage healing both histologically and morphologically, expressing the hyaline cartilage marker type II collagen [72]. In a rabbit model, OA was induced by ACLT for eight weeks and two million autologous BM-MSCs with a hyaluronan-based scaffold were delivered to the joint by intra-articular injection. Three to six months after surgery, the cartilage appeared significantly regenerative, expressing more type II collagen compared with the scaffold alone group. In addition, the cartilage matrix-degrading enzymes matrix metalloproteinase 1 and 3 (MMP-1, MMP-3) were decreased in the cell-treated group [73]. To track the injected MSCs, transgenic rats expressing dual luciferase (Luc) and LacZ were used in a rat massive meniscal defect model [74]. Twelve weeks after five million allogeneic Luc/LacZ$^+$ synovium-MSCs were injected, LacZ-positive regenerated menisci producing type II collagen were found, and the LacZ gene derived from MSCs was not found in any other organs except in synovium. In a spontaneous OA model in guinea pigs, seven million human commercial MSCs (Lonza, Basel, Switzerland) with hyaluronic acid (HA) were injected into the OA joint [75]. Five weeks after transplantation, partial cartilage regeneration with type II collagen expression was

Table 2 MSC-based treatment in pre-clinical experimental models of OA

Animal model	Animal species	Cell source	Scaffold	Dose/cells	Outcome	Reference
ACLT+ meniscectomy for 6 weeks	Goat	Allogeneic bone marrow	HA	2×10^6	Degeneration of the articular cartilage, osteophytic remodeling, and subchondral sclerosis were reduced in cell-treated joints.	Murphy et al. (2003) [24]
Partial-thickness cartilage defect model	Porcine	Autologous bone marrow	HA	9×10^5	At 12 weeks, the Wakitani scores showed marked improvement in the quality of the repair tissue seen in the MSC treated group.	Lee et al. (2007) [98]
Osteochondral (full-thickness) defect model	Rabbit	Allogeneic synovial	NA	1×10^7	The histological score of the treated group was consistently better at 4, 12, and 24 weeks than controls. The MSC suspension promoted cartilage regeneration.	Koga et al. (2008) [99]
Osteochondral defect model	Rabbit	Allogeneic bone marrow	OPF/GMP +TGF-β1	1×10^7	Defects were filled with hyaline cartilage-like tissue with zonal organization and intense glycosaminoglycan staining.	Guo et al. (2010) [100]
7 months old, spontaneous OA	Guinea pig	Commercial human MSC	HA	7×10^6	At 5 weeks post transplantation, partial cartilage repair was noted in the HA-MSC group with type II collagen around both residual chondrocytes and transplanted MSCs in the OA cartilage.	Sato et al. (2012) [75]
ACLT for 8 weeks	Rabbit	Allogeneic adipose	NA	$(1–2)\times10^6$	Treatment reduced matrix degrading enzymes and TNF-α in the cartilage matrix and inhibited MMP-1 and TNF-α expression in the synovial membrane and menisci.	Giovanna et al. (2013) [97]

noted in the cell-treated group.

These successful preclinical studies led to the initiation of many clinical trials (Table 3). The majority of these reports involved the use of autologous, culture-expanded BM-MSCs or A-MSCs. Notably, the majority of technical approaches used intra-articular injection to deliver the MSCs to the synovial fluid compartment using a hyaluronan scaffold, which is a major component of synovial fluid. The clinical reports listed in Table 3 tested cell injection doses from one to one hundred million cells in a single injection.

In a prospective, randomized, controlled clinical trial with two years follow-up, the microfracture with autologous BM-MSCs treatment group (28 patients) achieved significant improvements in the Tegner Lysholm and International Knee Documentation Committee (IKDC) scores. Magnetic resonance imaging (MRI) scans performed one year after the surgical intervention showed significantly better Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) scores for the cell-recipient group than the microfracture alone group (28 patients) [76]. In a pilot study of 12 OA patients with intra-articular injection of 40 million autologous expanded BM-MSCs, the patients showed a large improvement in algofunctional indices by one year, and MRI scanning exhibited a 27% decrease in poor cartilage areas with improvement of cartilage quality in 11 of the 12 patients [77]. Most recently, two clinical trials have attracted a lot of interest. In an Osiris Therapeutics Inc. funded, randomized, double-blinded, controlled clinical trial [25], 55 patients at seven institutions experienced a partial medial meniscectomy followed by injection with $(5–15)\times10^7$ allogeneic BM-MSCs and/or sodium hyaluronate as a vehicle control. At the two year follow-up, the meniscal volume achieved a 15% threshold in 24% of patients determined by quantitative MRI, indicating evidence of meniscus regeneration after treatment with allogeneic human BM-MSCs. Another intriguing proof-of-concept clinical trial in which patients with OA were treated with $(1–10)\times10^7$ autologous A-MSCs also demonstrated improved Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores at six months after injection in the 10×10^7 dose group [26]. Most importantly, the arthroscopy and histological assessments showed substantial thick, hyaline-like cartilage regeneration, suggesting that the intra-articular injection of A-MSCs regenerated hyaline-like articular cartilage without causing adverse events. All of these pre-clinical investigations and clinical trials demonstrated that the intra-articular injection of MSCs into an OA joint was not associated with apparent adverse events, but instead showed improved function, reduced pain, and regenerated hyaline-like cartilage in the affected joint.

Table 3 Clinical trials of MSC treatment for OA

Indications	Cell source	Dose/cells	Study design	Outcome	Reference
1 patient with degenerative joint disease	Autologous bone marrow	NA	24 weeks follow-up visit after intra-articular injection	The patient had statistically significant cartilage and meniscus growth by MRI, as well as increased range of motion and decreased modified VAS pain scores.	Centeno et al. (2008) [101]
50 patients with mild to moderate knee OA	Autologous cells	NA	Received MSC concentrate injection along with the arthroscopic debridement	The overall osteoarthritis outcome score, especially the quality of life, was improved.	Varma et al. (2010) [102]
4 patients with moderate to severe knee OA	Autologous bone marrow	$(8–9)\times10^6$	Intra-articular injection of cultured MSCs	The walking time and pain improved for 3 patients, and 1 patient remained unchanged.	Davatchi et al. (2011) [103]
12 patients with knee OA	Autologous infra-patellar fat pad	1.89×10^6	Intra-articular injection of cultured MSCs with PRP	The mean Lysholm score, Tegner activity scale, and VAS scores of patients in the study group improved significantly.	Koh et al. (2012) [104]
56 patients with unicompartmental knee OA	Autologous bone marrow	1.5×10^7	Intra-articular injection of cultured MSCs with hyaluronic acid 3 weeks after HTO and microfracture	The treatment was effective in improving both short-term clinical and MOCART outcomes.	Wong et al. (2013) [76]
12 patients with chronic knee pain	Autologous bone marrow	4×10^7	1 year follow-up visit after intra-articular injection	Patients exhibited rapid and progressive improvement of algofunctional indices that approached 65% to 78% after 1 year.	Orozco et al. (2013) [77]
18 patients with knee OA	Autologous adipose	$(1–10)\times10^7$	The phase I study consisted of intra-articular injection of 3 dose-escalation cohorts. The phase II study included 9 patients receiving the high-dose and their 6 months follow-up visit	Intra-articular injection of the high dose improved function and pain of the knee joint without causing adverse events, and reduced cartilage defects by regeneration of hyaline-like articular cartilage.	Jo et al. (2014) [26]
55 patients with partial medial meniscectomy	Allogeneic bone marrow	5×10^7	A single superolateral knee injection of cultured MSCs 7 to 10 days after the meniscectomy	Treated patients had a significant reduction in pain, along with significantly increased meniscal volume.	Vangsness et al. (2014) [25]

5 Potential mechanisms involved in OA progression and MSC treatment

In OA joints, the synovium appears to have at least two crucial roles in the development and progression of OA. On one hand, synovium might be the target of effective repair responses involving endogenous MSCs. On the other hand, the resident cell population within the synovium affects the homeostasis of the joint, and can initiate degenerative changes in OA [78,79]. The infiltration of $CD4^+$ T cells and $CD68^+$ macrophages is essentially increased in the synovium during early-stage compared with late-stage OA [80]. Moreover, cytokines, including interleukin-1 beta (IL-1β) and TNF-α, and chemokines (CCL 19 and monocyte chemotactic protein 1) may be released from the synovium, stimulating articular chondrocytes by activating various cell surface receptors, including Toll-like receptors (TLRs), and thereby promoting cartilage catabolism and inhibiting matrix synthesis [81]. All these data suggest that synovial inflammation is a feature in the early-stages of OA and might initiate the degenerative cascades that result in tissue destruction.

Chondrocytes also play an important role in OA progression via several signaling pathways. Chondrocytes can be activated through the nuclear factor kappa B (NF-κB), stress-induced, and mitogen-activated protein kinase (MAPK) pathways by mechanical and inflammatory stimuli to express cytokine and chemokine receptors, MMPs, cyclooxygenase 2 (COX-2), and IL-1 [82]. Cartilage matrix-degrading enzymes secreted by chondrocytes and synovial cells include aggrecanases and collagenases. Matrix degradation during early-stage OA may be caused by aggrecanases, MMP-3, and a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS-5) activities, followed by increased activity of collagenase MMP-13, which is highly efficient at degrading type II collagen [83]. These results were confirmed in global knockout *Adamts-5*$^{-/-}$ mice, which exhibited protection against OA progression and in global knockout *Mmp-13*$^{-/-}$ mice, which showed inhibition of cartilage erosion [84,85]. Recently, the canonical Wnt/β-catenin signaling pathway was suggested to have dual effects on cartilage destruction during OA progression. A global knockout *Frzb*$^{-/-}$, a Wnt antagonist, exhibited greater cartilage loss than wild type controls, and the increased cartilage damage was associated with increased levels of β-catenin-dependent Wnt signaling and matrix metalloproteinase 3 [86]. Another experiment also found that Wnt antagonist *Dkk-1* chondrocyte-specific transgenic mice, *Col2α1-Dkk1*, had significantly mitigated surgery-induced OA because of decreased *Mmp13* and *Adamts4* expression compared with their wild-type littermates, while Wnt-3a, an agonist of Wnt, induced *Mmp13* and *Adamts4* expression in chondrocytes in primary culture [87]. However, further investigation found that Wnt-3a could block IL-1β-induced MMP-1 and MMP-13 expression, while co-incubation with Dkk-1 increased IL-1β-induced expression of MMPs. This indicates a negative feedback loop wherein Wnt/β-catenin signaling in human chondrocytes played an anti-catabolic role by preventing MMP expression induced by IL-1β [88]. Another investigation also found that *SOST*, a potent inhibitor of canonic Wnt signaling that binds to Wnt receptor Low-density-lipoprotein receptor-related protein 5/6 (LRP5/6), inhibited further degradation of OA cartilage [89]. More recently, bone morphogenetic protein 2 (BMP-2) signaling was found to increase β-catenin nuclear translocation, LRP-5 expression, MMP levels (MMP-9, MMP-13, and MMP 14), ADAMTS-5, and collagen X expression. The BMP-2-induced LRP-5 up-regulation was mediated by Smad1/5/8 binding on the LRP-5 promoter, which contributed to the hypertrophy of OA chondrocytes via crosstalk with canonical Wnt/β-catenin signaling [90].

Whether subchondral bone thickening precedes fibrillation of the cartilage or not remains controversial. Recently, articular chondrocytes isolated from normal joints were found to prohibit normal subchondral bone osteoblastogenesis, whereas chondrocytes isolated from OA joints strengthened subchondral bone osteoblastogenesis by significantly activating ERK 1/2 phosphorylation in co-culture, indicating that OA chondrocytes may alter subchondral bone osteoblastogenesis via the MAPK signaling pathways [91]. Interestingly, OA subchondral bone osteoblasts were also able to increase articular chondrocyte differentiation by inhibiting p38 phosphorylation and inducing ERK 1/2 phosphorylation [92].

In terms of the mechanism of action of MSC therapy for OA, a recent investigation found that injection of human MSCs promoted rat meniscal regeneration by expressing rat collagen II, whereas a hedgehog antagonist prohibited, and a hedgehog agonist promoted rat collagen II expression. These data suggest that intra-articular injection of hMSCs repaired cartilage by activating the Indian hedgehog signaling pathway [93]. The expression of Notch-1 was also found in MSCs from healthy and OA articular cartilage, but the numbers of Notch-1-positive MSCs were much larger when isolated from OA articular cartilage than healthy samples [94], indicating that Notch signaling is overexpressed during OA progression and that intra-articular therapy with normal MSCs down-regulates the Notch signaling pathway. The transient receptor potential vanilloid 4 (TRPV 4) ion channel, a Ca^{2+}-preferred cation channel, was also found to play an important role in the progression of OA in mice using a global *Trpv4* knock out [95]. Further investigations have demonstrated that BM-MSCs from *Trpv4*$^{-/-}$ mice showed decreased adipogenesis and osteogenesis

phenotypes, whereas A-MSCs from *Trpv4*$^{-/-}$ mice exhibited increased adipogenesis and osteogenesis and decreased chondrogenesis compared with those in wild type controls, indicating that Trpv4 plays a chondroprotective role during OA progression [96]. Recent investigations found that in OA animal models, intra-articular injection of MSCs decreased articular cartilage erosion, probably by inhibiting MMP-13, TNF-α, and IL-1β expression [75,97]. Because MMP-13, TNF-α, and IL-1β expressions were associated with Wnt/β-catenin, MAPK, and BMP signaling based on the studies mentioned above, we inferred that these signals may also be involved in the effects of MSC treatment on OA (Figure 1).

6 Conclusion

In conclusion, OA is a disease characterized by progressive and irreversible destruction of the entire joint structure. OA seems to occur because of changes in the quantity, phenotype, and differentiation potential of resident mesenchymal cells. Nevertheless, current therapies, including conservative treatments and surgery, result in poor clinical outcomes with no cartilage repair. Successful pre-clinical results using intra-articular injection of MSCs provide the impetus for considering injection of MSCs as a useful therapy for this obstinate disease. Recent clinical studies have demonstrated exciting results with hyaline-like cartilage regeneration accompanied by pain relief, joint function improvement, and amelioration of the joint's health condition. Several signaling pathways have been found to be altered, along with changes in the functions, of joint-resident MSCs during OA progression, suggesting the necessity of exogenous MSC transplantation.

However, there are still a large number of unanswered questions, some of which are exigent. For example, does MSC treatment repair the cartilage degradation, synovitis, and subchondral bone sclerosis directly or through paracrine effects? Which factors released by MSCs protect cartilage from degradation or trigger regeneration or repair of cartilage? What are the mechanisms of the cross-talk and feedback between injected MSCs and synovium, articular cartilage, and subchondral bone, and which are relevant to directing disease progression and remission? Answering these questions will most likely require systemic approaches that apply molecular level genomics, epigenetics, proteomics, and metabolomics to investigate the mechanisms of action of MSCs during OA treatment.

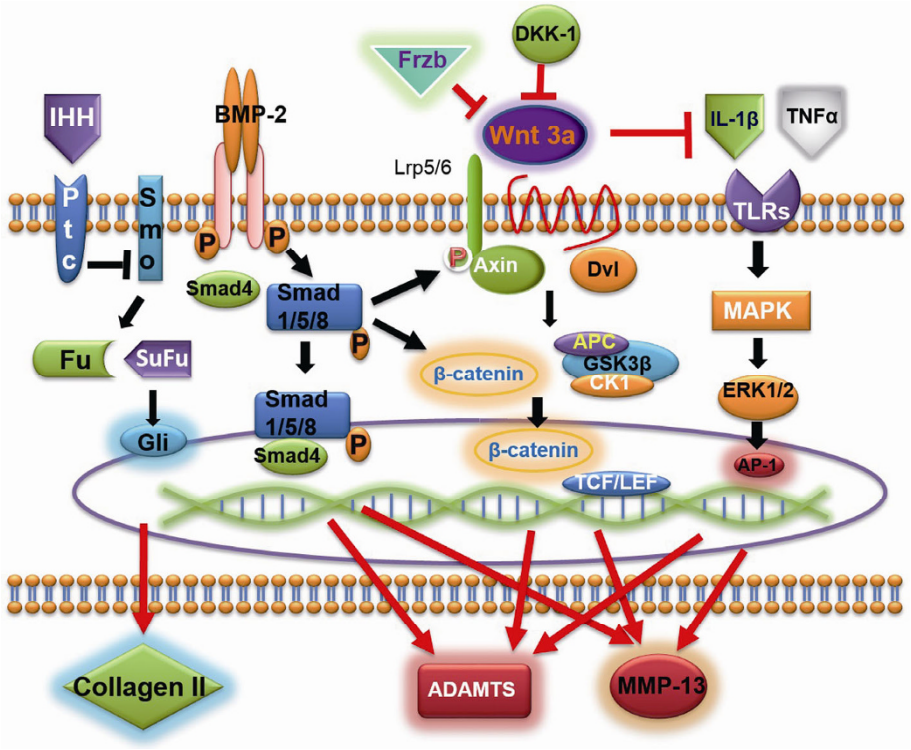

Figure 1 Potential mechanisms involved in OA progression and MSC treatment. Intra-articular injection of hMSCs promotes rat meniscal regeneration by expression of rat collagen II via Indian Hedgehog (IHH) signaling, whereas Smoothened IHH antagonist cyclopamine prohibited, and Smoothened IHH agonist SAG promoted, rat collagen II expression. BMP-2 signaling may not only increase MMP levels directly, but also increase β-catenin translocation via crosstalk with canonical Wnt/β-catenin signaling. Wnt-3a, an agonist of Wnt, may induce MMP13 and ADAMTS-4 expression, while *Frzb*$^{-/-}$ mice exhibited cartilage loss and *Dkk1* transgenics showed cartilage gain, though both were Wnt antagonists. MAPK signaling may also activate chondrocytes to secrete ADAMTS and MMPs.

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